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ORIGINAL ARTICLE

P73 G4C14-to-A4T14 polymorphism is associated with survival in advanced non-small cell lung cancer patients

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Abstract

Background: *p73*, a structural and functional homolog of *p53*, plays an important role in modulating cell cycle arrest. This study investigated the association between *p73* G4C14-to-A4T14 polymorphism and survival outcomes in a Chinese population of advanced non-small cell lung cancer (NSCLC) patients treated with platinum agents.

Methods: The *p73* G4C14-to-A4T14 polymorphism was genotyped using DNA from blood samples of advanced NSCLC patients (642 in the discovery set and 330 in the replication set). The relationship of the *p73* G4C14-to-A4T14 polymorphism with clinical outcomes was analyzed.

Results: Compared with the GC/GC genotype, the genotypes containing AT allele (GC/AT + AT/AT genotypes) were associated with significantly prolonged overall survival ($P = 0.040$) in the discovery set and after pooling results from the replication set. Stratification analysis revealed that the association was more pronounced in subjects who were older ($P = 0.001$), male ($P = 0.007$), smokers ($P = 0.006$), had a low Eastern Cooperative Oncology Group performance status ($P = 0.001$), in tumor node metastasis stage IV ($P = 0.008$), and with adenocarcinoma ($P = 0.002$). The objective response rates of patients with GC/AT + AT/AT genotypes were statistically higher than those with the GC/GC genotype ($P = 0.047$).

Conclusion: Our findings suggest that the *p73* G4C14-to-A4T14 polymorphism may be related to survival outcome in advanced NSCLC patients.

Introduction

Non-small-cell lung cancer (NSCLC), accounts for approximately 85% of primary lung cancers and remains the leading cause of cancer-related death worldwide. Despite the encouraging improvement of treatment methods over the last decades, the five-year survival rate is still low, which is mainly attributed to the large proportion of advanced cases at the time of diagnosis.[1] Combination therapy based on platinum agents has been the most common form of treatment for advanced NSCLC, regardless of various therapy responses between patients.[2] However, the major problem is the optimization of treatment options, which could help clinicians determine which patients will benefit from which therapy.

The *P73* gene, a structural and functional homolog of the *p53* gene, plays a crucial role in the presence of DNA damage induced by platinum-based chemotherapy.[3] The *p73* G4C14-to-A4T14 polymorphism consists of two single nucleotide polymorphisms (SNPs, rs2273953 and rs1801173), which are in complete linkage disequilibrium,

located at positions 4 (G→A) and 14 (C→T) in the 5′ untranslated region (UTR) of exon 2, just upstream of the initiating AUG of the *p73* gene. It has been shown that the GC to AT change may form a stem-loop structure and possibly affect the translation efficiency of *p73*.[4] An increasing number of studies have investigated the relationship between the *p73* G4C14-to-A4T14 polymorphism and the susceptibility of various cancers, including lung, breast, esophageal, prostate, cervical, and gastric carcinoma in different ethnic populations.[5–10] In addition, evidence has also indicated that the expression level of the *p73* gene is a non-ignorable factor of chemosensitivity in human tumors.[11]

However, in spite of the well-known impact of the *p73* G4C14-to-A4T14 polymorphism on cancer development, its potential role in chemotherapeutic response and prognosis of NSCLC has not been fully investigated. To further test the association, we performed a two-stage association analysis for this validated polymorphism by conducting a discovery cohort with 642 advanced NSCLC patients who received platinum-based chemotherapy followed by further replication in an independent replication cohort with 330 patients in a Chinese population.

Methods

Study population and follow-up

The discovery set included 642 cases with confirmed late-stage (III–IV) NSCLC who had received platinum-based chemotherapy between March 2005 and January 2010 in the oncological departments of Shanghai Zhongshan Hospital, Shanghai Chest Hospital, and Shanghai Changhai Hospital. Positive hits from the discovery set were validated in patients with advanced NSCLC from an independent replication cohort. This dataset included 330 advanced NSCLC cases from Shanghai Pulmonary Hospital between June 2010 and May 2013. Blood samples from all subjects were collected at the time of diagnosis, prior to chemotherapy treatment. All subjects provided written informed consent and the medical ethics committee of each participating institution approved the study.

Follow-up was performed every three months from the time of enrollment until death or the last follow-up. Data of all cases were collected retrospectively from the medical records and databases of each hospital. The definition of non-smokers used was described in a previous study.[12] Overall survival (OS) was defined as the period from receipt of chemotherapy to the time of death or last follow-up. Progression-free survival (PFS) was defined as the duration from the first treatment to the date of disease progression, death or last follow-up. Therapeutic response was assessed after the first two or three cycles and determined by Response Evaluation Criteria in Solid Tumors version 1.1.[13] The disease control rate (DCR) included complete response (CR), partial response (PR) and stable disease (SD). The objective response rate (ORR) consisted of complete response (CR) and partial response (PR).

Chemotherapy regimens

All participants received first-line platinum-based chemotherapy; the detailed chemotherapeutic regimens have previously been described.[12]

Genotype analysis

Blood samples were collected from each participant and genomic DNA was extracted using the Human Whole Blood Genomic DNA Extraction Kit (Qiagen, Valencia, CA, USA). We analyzed samples for the *p73* G4C14-to-A4T14 polymorphism using the TaqMan Pre-Designed SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. The PCR primers used for amplifying *p73* G4C14-A4T14 were as follows: 5′-CAGGAGGACAGAGCACGAGTT-3′ (forward) and 5′-TGATGAGGGTGGCTAAGGCTA-3′ (reverse). Approximately 15% of the samples were randomly selected for repeat genotyping by a different investigator, and the results were entirely concordant.

Statistical analysis

The distribution of selected variables and *p73* genotype frequencies between the discovery and replication sets were evaluated using the χ^2 test. Hardy–Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies. Survival curves were computed according to Kaplan–Meier curves. Univariate analysis was conducted using Cox's proportional hazard model to validate the significant variables related to survival. Multivariate analysis was then performed using variables with a univariate $P < 0.1$. For chemotherapeutic response, unconditional multivariate logistic regression analysis was performed to estimate odds ratios (ORs), along with the corresponding 95% confidence intervals (CIs) for *p73* genotypes. All statistical analyses were accomplished using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and P values <0.05 were considered statistically significant.

Results

Patient characteristics

The demographic and clinical characteristics of the patients in the two study cohorts are presented in Table 1 . In the discovery set, 456 (71.0%) patients were male and 382 (59.5%) were smokers. All patients had advanced inoperable NSCLC,

Table 1 Basic patient characteristics

Variables	Discovery set (N, %) (*n* = 642)	Replication set (N, %) (*n* = 330)	χ^2	*P**
Age (years)				
<58	333 (51.9)	171 (51.8)	0.001	0.988
≥58	309 (48.1)	159 (48.2)		
Gender				
Male	456 (71.0)	234 (70.9)	0.001	0.969
Female	186 (29.0)	96 (29.1)		
Smoking history				
Non-smokers	260 (40.5)	144 (43.6)	0.884	0.347
Smokers	382 (59.5)	186 (56.4)		
ECOG PS				
0–1	593 (92.4)	297 (90.0)	1.582	0.209
2	49 (7.6)	33 (10.0)		
Chemotherapy				
NP/NC	236 (36.8)	115 (34.8)	1.126	0.771
GP/GC	174 (27.1)	95 (28.8)		
TP/TC	192 (29.9)	95 (28.8)		
DP/DC	40 (6.2)	25 (7.6)		
TNM stage				
III	262 (40.8)	119 (36.1)	2.063	0.151
IV	380 (59.2)	211 (63.9)		
Tumor histology				
Adeno	398 (62.0)	213 (64.5)	2.057	0.358
SQC	147 (22.9)	66 (20.0)		
Others	97 (15.1)	51 (15.5)		

**P*-values derived from χ^2 test. Adeno, adenocarcinoma; DP/DC, carboplatin or cisplatin plus docetaxel; ECOG PS, Eastern Cooperative Oncology Group performance status; GP/GC, carboplatin or cisplatin plus gemcitabine; NP/NC, carboplatin or cisplatin plus vinorelbine; SQC, squamous cell carcinoma; TNM, tumor node metastasis; TP/TC, carboplatin or cisplatin plus paclitaxel.

with 40.8% in stage III and 59.2% in stage IV. The median OS and PFS of all patients were 19.27 (95% CI: 17.64–20.89) and 10.07 months (95% CI: 8.61–11.53), respectively. Patients with tumor node metastasis (TNM) stage IV and infrequent histological subtypes had significantly worse OS ($P = 0.012$ and $P = 0.027$, respectively) compared with patients with TNM stage III and adenocarcinoma (Fig 1a,b). In addition, Eastern Cooperative Oncology Group (ECOG) performance status was related to PFS and patients with higher scores showed a higher risk of recurrence or metastasis ($P = 0.006$; Fig 1c), whereas other clinical factors were not independent prognostic factors (Table 2).

Among the 330 cases in the replication set, 234 (70.9%) were men and 186 (56.4%) were smokers, with 36.1% in stage III and 63.9% in stage IV disease. TNM stage and tumor histology also showed significant associations with OS, similar to the discovery set (Table 3). There were no statistically significant differences in variables between the discovery and replication sets (Table 1).

Survival analysis

Discovery set

All genotype frequencies for *p73* G4C14-to-A4T14 were in Hardy–Weinberg equilibrium ($P > 0.05$). There was no significant difference in the genotype distributions of *p73* G4C14-to-A4T14 according to clinical factors (Table 3). We classified this polymorphism by models including genotypic, dominant, recessive, and additive. The results demonstrated that individuals with the AT/AT genotype have prolonged OS compared with GC/GC carriers (adjusted hazard ratio [aHR] = 0.65, $P = 0.035$), whereas heterozygotes showed no significance after adjusting for selected variables. In addition, patients carrying the AT allele (GC/AT or AT/AT) had significantly increased OS in the dominant model (for GC/AT + AT/AT genotype HR 0.82; $P = 0.040$). Kaplan–Meier curves also indicated these results (log-rank test for the genotypic model $P = 0.019$, for the dominant model $P = 0.021$; Fig 1d,e; Table 2). However, none of genotypes showed a significant relationship with PFS (data not shown).

Replication set and pooled analysis

To validate the association of *p73* G4C14-to-A4T14 with OS, another independent replication set with 330 advanced NSCLC cases was performed. In this second group, the *p73* G4C14-to-A4T14 genotypes showed a similar trend of relationship with survival. We concluded that carriers of the AT/AT genotype were significantly associated with

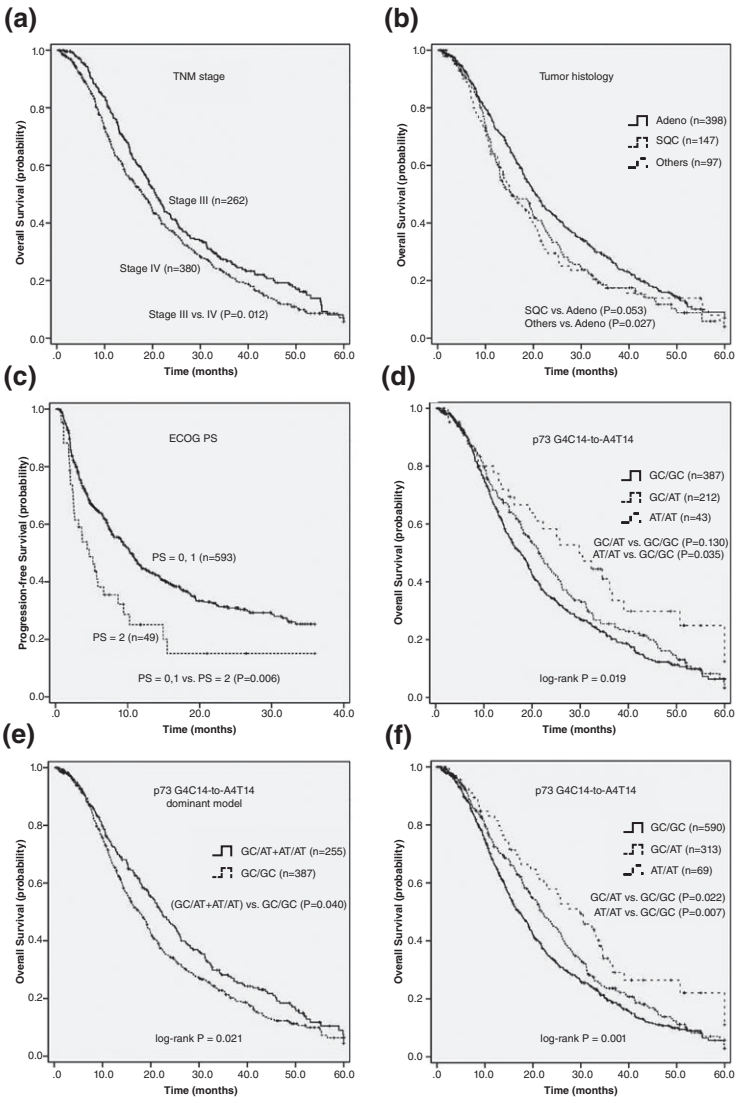

Figure 1 Survival analysis. Kaplan–Meier curves of (**a**) tumor node metastasis (TNM) stage and overall survival (OS), (**b**) tumor histology and OS, (**c**) Eastern Cooperative Oncology Group performance status (ECOG PS) and progression-free survival (PFS), (**d**) *p73* G4C14-to-A4T14 and OS, and (**e**) *p73* G4C14-to-A4T14 dominant model and OS in the discovery set. (**f**) *p73* G4C14-to-A4T14 and OS in pooled populations.

increased OS compared with the GC/GC genotype (aHR = 0.64, $P = 0.040$). The dominant ($P = 0.014$) and additive models ($P = 0.015$) of *p73* G4C14-to-A4T14 also showed a statistically significant association (Table 4).

Further pooled analysis of the two cohorts verified the previously observed association of the *p73* G4C14-to-A4T14 polymorphism with OS. Kaplan–Meier curves and log-rank tests showed that both the AT/AT variant homozygotes and the GC/AT heterozygotes were significantly correlated with prolonged OS compared with the GC/GC homozygotes in the 972 NSCLC patients ($P = 0.001$; Fig 1 f). Univariate Cox regression analysis also confirmed this association (for GC/AT + AT/AT genotype HR 0.80; $P = 0.003$; for AT/AT genotype HR 0.69; $P = 0.032$; for additive model HR 0.82, $P = 0.001$) after adjusting for clinical variables (Table 3). Furthermore, we included age (≥58 vs. <58), TNM stage (TNM IV vs. TNM III), tumor histology (adenocarcinoma, squamous, or others), and the *p73* G4C14-to-A4T14 dominant and recessive models in multivariate Cox regression analysis. The results suggested that TNM stage ($P = 0.004$), tumor histology ($P = 0.022$), and dominant model ($P = 0.017$) were independent predictive factors for OS (Table 5).

Stratification analysis

To better understand the potential impact of the *p73* G4C14-to-A4T14 polymorphism on survival in NSCLC patients, we performed subgroup analysis stratified by confounding variables in the pooled populations. Because the AT/AT genotype was relatively infrequent, we combined it with the GC/AT genotype for further examination. As shown in Table 6 , the favorable effect of the *p73* combined genotypes (GC/AT + AT/AT) was more evident in patients

Table 2 Survival analysis in the discovery set

Variables	N (%)	OS (95% CI) (m)†	$P_{L\text{-}R}$	aHR (95% CI)‡	*P*‡
Age (years)					
<58	333 (51.9)	20.87 (18.73–23.00)	0.028	Reference	
≥58	309 (48.1)	16.40 (14.04–18.76)		1.17 (0.97–1.41)	0.095
Gender					
Male	456 (71.0)	18.67 (16.54–20.79)	0.049	Reference	
Female	186 (29.0)	20.87 (17.29–24.45)		0.88 (0.65–1.18)	0.375
Smoking history					
Non-smokers	260 (40.5)	20.23 (17.60–22.87)	0.084	Reference	
Smokers	382 (59.5)	18.30 (15.93–20.67)		1.03 (0.78–1.34)	0.858
ECOG PS					
0–1	593 (92.4)	19.37 (17.74–21.00)	0.121	Reference	
2	49 (7.6)	17.77 (8.76–26.77)		1.24 (0.89–1.73)	0.206
Chemotherapy					
NP/NC	236 (36.8)	19.17 (16.30–22.03)	0.447	Reference	
GP/GC	174 (27.1)	19.27 (16.32–22.21)		0.88 (0.70–1.11)	0.279
TP/TC	192 (29.9)	19.03 (15.99–22.08)		1.03 (0.83–1.29)	0.779
DP/DC	40 (6.2)	22.00 (18.41–25.59)		0.84 (0.58–1.23)	0.381
TNM stage					
III	262 (40.8)	20.67 (18.53–22.80)	0.029	Reference	
IV	380 (59.2)	17.80 (15.43–20.17)		1.28 (1.06–1.54)	0.012
Tumor histology					
Adeno	398 (62.0)	20.37 (18.31–22.43)	0.030	Reference	
SQC	147 (22.9)	15.27 (10.63–19.90)		1.26 (0.99–1.60)	0.053
Others	97 (15.1)	15.30 (11.02–19.58)		1.35 (1.03–1.76)	0.027
P73 G4C14-to-A4T14					
GC/GC	387 (60.3)	17.67 (15.71–19.62)	0.019	Reference	
GC/AT	212 (33.0)	21.37 (18.42–24.31)		0.86 (0.71–1.05)	0.130
AT/AT	43 (6.7)	29.80 (19.38–40.22)		0.65 (0.43–0.97)	0.035
Dominant					
GC/AT + AT/AT	255 (39.7)	22.30 (19.08–25.52)	0.021	0.82 (0.68–0.99)	0.040
GC/GC	387 (60.3)	17.67 (15.71–19.62)		Ref.	
Recessive					
AT/AT	43 (6.7)	29.80 (19.38–40.22)	0.021	0.69 (0.46–1.02)	0.063
GC/GC +GC/AT	599 (93.3)	19.03 (17.40–20.66)		Reference	
Additive	NA	NA	NA	0.83 (0.72–0.97)	0.017

†Survival derived from Kaplan–Meier analysis. ‡Hazard ratios (HRs), 95% confidence intervals (CIs) and their corresponding *P* values were calculated using univariate Cox proportional hazard models. Adeno, adenocarcinoma; aHR, adjusted hazard ratio; ECOG PS, Eastern Cooperative Oncology Group performance status; GP/GC, carboplatin or cisplatin plus gemcitabine; m, months; NA, not available; NP/NC, carboplatin or cisplatin plus vinorelbine; OS, overall survival; $P_{L\text{-}R}$, Log-Rank *P*; SQC, squamous cell carcinoma; TNM, tumor node metastasis; TP/TC, carboplatin or cisplatin plus paclitaxel.

who were older (≥58 years) at diagnosis ($P = 0.001$), male ($P = 0.007$), smokers ($P = 0.006$), had a low ECOG performance status (0–1; $P = 0.001$), in TNM stage IV ($P = 0.008$), and with adenocarcinoma ($P = 0.002$). However, we did not find any interaction between the *p73* variant genotypes and chemotherapeutic regimens for overall survival in NSCLC patients.

P73 G4C14-to-A4T14 and chemotherapy efficacy

The chemotherapeutic response of patients was assessed in the pooled populations. Disease control was noted in 761 (78.3%) patients, and objective response was achieved in 163 (16.8%). A marginally significant association with ORR other DCR for *p73* G4C14-to-A4T14 was manifested by multivariate logistic regression analysis in the 972 NSCLC cases (for GC/AT + AT/AT genotype OR, 0.69; $P = 0.047$; Table 7).

Discussion

The principal finding of the present study is that the *p73* G4C14-to-A4T14 polymorphism may be related to survival outcomes in advanced NSCLC patients who receive platinum-based chemotherapy. Given the role of *p73* as an important regulator of cell cycle and DNA repair, it is

Table 3 Distribution of *p73* G4C14-to-A4T14 genotypes according to clinical factors

	Discovery set, n (%)					Replication set, n (%)				
Variables	GC/GC (*n* = 387)	GC/AT (*n* = 212)	AT/AT (*n* = 43)	χ^2	*P**	GC/GC (*n* = 203)	GC/AT (*n* = 101)	AT/AT (*n* = 26)	χ^2	*P**
Age (years)										
<58	202 (60.7)	112 (33.6)	19 (5.7)	1.112	0.574	102 (59.6)	54 (31.6)	15 (8.8)	0.670	0.715
≥58	185 (59.9)	100 (32.4)	24 (7.8)			101 (63.5)	47 (29.6)	11 (6.9)		
Gender										
Male	280 (61.4)	148 (32.5)	28 (6.1)	1.112	0.545	145 (62.0)	73 (31.2)	16 (6.8)	1.225	0.542
Female	107 (57.5)	64 (34.4)	15 (8.1)			58 (60.4)	28 (29.2)	10 (10.4)		
Smoking history										
Non-smokers	150 (57.7)	90 (34.6)	20 (7.7)	1.467	0.480	84 (58.3)	48 (33.3)	12 (8.3)	1.108	0.575
Smokers	237 (62.0)	122 (31.9)	23 (6.0)			119 (64.0)	53 (28.5)	14 (7.5)		
ECOG PS										
0–1	355 (59.9)	196 (33.1)	42 (7.1)	1.942	0.379	181 (60.9)	92 (31.0)	24 (8.1)	0.445	0.800
2	32 (65.3)	16 (32.7)	1 (2.0)			22 (66.7)	9 (27.3)	2 (6.1)		
Chemotherapy										
NP/NC	137 (58.1)	83 (35.2)	16 (6.8)	6.962	0.324	74 (64.3)	31 (27.0)	10 (8.7)	9.509	0.147
GP/GC	101 (58.0)	59 (33.9)	14 (8.0)			54 (56.8)	33 (34.7)	8 (8.4)		
TP/TC	127 (66.1)	57 (29.7)	8 (4.2)			57 (60.0)	34 (35.8)	4 (4.2)		
DP/DC	22 (55.0)	13 (32.5)	5 (12.5)			18 (72.0)	3 (12.0)	4 (16.0)		
TNM stage										
III	161 (61.5)	78 (29.8)	23 (8.8)	4.379	0.112	74 (62.2)	33 (27.7)	12 (10.1)	1.665	0.435
IV	226 (59.5)	134 (35.3)	20 (5.3)			129 (61.1)	68 (32.2)	14 (6.6)		
Tumor histology										
Adeno	236 (59.3)	133 (33.4)	29 (7.3)	1.307	0.860	130 (59.1)	69 (31.4)	21 (9.5)	3.682	0.451
SQC	93 (63.3)	45 (30.6)	9 (6.1)			42 (63.6)	21 (31.8)	3 (4.5)		
Others	58 (59.8)	34 (35.1)	5 (5.2)			31 (70.5)	11 (25.0)	2 (4.5)		

**P* values derived from χ^2 test. Adeno, adenocarcinoma; ECOG PS, Eastern Cooperative Oncology Group performance status; GP/GC, carboplatin or cisplatin plus gemcitabine; m, months; NP/NC, carboplatin or cisplatin plus vinorelbine; SQC, squamous cell carcinoma; TNM, tumor node metastasis; TP/TC, carboplatin or cisplatin plus paclitaxel.

biologically plausible that this well known SNP may potentially modulate the chemotherapy efficacy of DNA-damaging anti-cancer drugs, including platinum agents.

DNA damage in the G1 phase induced by platinum agents leads to the activation of cell cycle checkpoints, such as the *p53* tumor suppressor, resulting in either G1 arrest or programmed cell death.[14] Like *p53*, *p73* has also been shown to respond to DNA damage, causing induction of cell cycle arrest or apoptosis.[15] Because genetic polymorphisms in DNA repair genes, including the *p53* Arg72Pro polymorphism, they potentially influence the activity of chemotherapeutic agents. We hypothesized that *p73* genetic polymorphisms could also contribute to the individual variability of drug response. Two landmark articles showed that *p73* is an important determinant of chemosensitivity in humans, and its function is highly integrated with that of *p53*.[16,17] Several other articles have confirmed the importance of *p73* expression in the prediction of tumor chemosensitivity and cancer prognosis by studying different tumor types.[18–21] The regulatory mechanisms are primarily linked to posttranslational modifications and protein-protein interactions involving both signaling molecules and transcription factors. Indeed, mechanical behavior may be complex and variable, as it is dependent on the specific drugs and tissues involved. Thus, research on the genetic variants of the *p73* gene relevant to DNA damage may contribute to fully understanding or predicting the effect of chemotherapy.

Recently, an increasing number of studies have suggested that this validated polymorphism of *p73* not only influences the development, but also has an impact on the progression and prognosis of various cancers. For example, Carastro *et al.* demonstrated that *p73* G4C14-to-A4T14 has a significant inverse relationship with aggressiveness and a marginal association with overall death in prostate cancer.[22] Pfeifer *et al.* reported that colorectal cancer patients with the AT allele had a better prognosis than those with the GC/GC genotype.[23] Lee *et al.* concluded that the *p73* GC/AT genotype is associated with increased risk and survival of colorectal cancer in a Korean population.[24] Liu *et al.* demonstrated the combined effect of genetic polymorphisms in the *p53*, *p73*, and *MDM2* genes on NSCLC survival.[25] However, the fairly small sample size and marginal significance mean that these results should

Table 4 Survival analysis in the replication set and pooled populations

	Replication set					Pooled populations				
Variables	N (%)	OS (95% CI) (m)†	$P_{L\text{-}R}$	aHR (95%CI)‡	*P*‡	N (%)	OS (95% CI) (m)†	$P_{L\text{-}R}$	aHR (95% CI)‡	*P*‡
Age (years)										
<58	171 (51.8)	21.87 (17.35–26.38)	0.038	Ref.		504 (51.9)	21.30 (19.25–23.35)	0.014	Reference	
≥58	159 (48.2)	17.57 (14.67–20.47)		1.33 (1.02–1.741)	0.072	468 (48.1)	17.17 (15.30–19.03)		1.20 (1.03–1.40)	0.055
Gender										
Male	234 (70.9)	17.93 (15.31–20.55)	0.063	Ref.		690 (71.0)	18.27 (16.64–19.89)	0.057	Reference	
Female	96 (29.1)	22.50 (16.47–28.53)		0.85 (0.60–1.18)	0.326	282 (29.0)	21.43 (17.95–24.92)		0.87 (0.69–1.10)	0.257
Smoking history										
Non-smokers	144 (43.6)	22.50 (17.87–27.13)	0.125	Ref.		404 (41.6)	21.03 (18.69–23.38)	0.025	Reference	
Smokers	186 (56.4)	17.57 (15.13–20.00)		1.18 (0.88–1.57)	0.269	568 (58.4)	17.93 (16.23–19.64)		1.05 (0.85–1.29)	0.412
ECOG PS										
0–1	297 (90.0)	19.40 (16.21–22.59)	0.482	Ref.		890 (91.6)	19.40 (17.93–20.87)	0.081	Reference	
2	33 (10.0)	18.00 (7.13–28.87)		1.26 (0.80–1.89)	0.312	82 (8.4)	17.90 (11.14–24.66)		1.22 (0.94–1.59)	0.134
Chemotherapy										
NP/NC	115 (34.8)	17.87 (13.39–22.34)	0.687	Ref.		351 (36.1)	18.63 (16.14–21.13)	0.399	Reference	
GP/GC	95 (28.8)	19.07 (13.67–24.46)		1.16 (0.84–1.60)	0.372	269 (27.7)	19.07 (16.37–21.76)		0.95 (0.79–1.14)	0.549
TP/TC	95 (28.8)	19.83 (15.48–24.19)		1.07 (0.78–1.48)	0.672	287 (29.5)	19.27 (17.25–21.29)		1.01 (0.85–1.22)	0.881
DP/DC	25 (7.6)	20.90 (11.11–30.69)		0.77 (0.42–1.40)	0.389	65 (6.7)	22.00 (18.01–25.99)		0.81 (0.59–1.12)	0.199
TNM stage										
III	119 (36.1)	21.43 (17.43–25.44)	0.018	Ref.		381 (39.2)	21.03 (19.06–23.01)	0.012	Reference	
IV	211 (63.9)	17.87 (14.72–21.02)		1.39 (1.06–1.834)	0.022	591 (60.8)	17.87 (16.04–19.70)		1.30 (1.12–1.52)	0.006
Tumor histology										
Adeno	213 (64.5)	20.67 (17.13–24.20)	0.081	Ref.		618 (63.6)	20.57 (18.71–22.43)	0.007	Reference	
SQC	66 (20.0)	17.57 (12.86–22.27)		1.27 (0.89–1.68)	0.192	213 (21.9)	16.63 (13.12–20.15)		1.16 (0.96–1.42)	0.140
Others	51 (15.5)	18.00 (14.48–21.53)		1.58 (1.08–2.32)	0.019	141 (14.5)	16.40 (13.11–19.69)		1.37 (1.10–1.70)	0.010
P73 G4C14-to-A4T14										
GC/GC	203 (61.5)	16.37 (14.23–18.50)	0.035	Ref.		590 (60.7)	17.00 (15.36–18.64)	0.001	Reference	
GC/AT	101 (30.6)	24.03 (18.76–29.31)		0.74 (0.55–0.99)	0.083	313 (32.2)	21.87 (19.08–24.66)		0.83 (0.71–0.97)	0.022
AT/AT	26 (7.9)	27.43 (15.97–38.90)		0.64 (0.38–1.06)	0.040	69 (7.1)	29.80 (22.32–37.28)		0.65 (0.47–0.89)	0.007
Dominant										
GC/AT + AT/AT	127 (38.5)	25.13 (20.66–29.61)	0.015	0.72 (0.55–0.94)	0.014	382 (39.3)	22.63 (20.04–25.23)	0.001	0.80 (0.68–0.93)	0.003
GC/GC	203 (61.5)	16.37 (14.23–18.50)		Ref.		590 (60.7)	17.00 (15.36–18.64)		Reference	
Recessive										
AT/AT	26 (7.9)	32.83 (22.11–43.56)	0.083	0.70 (0.42–1.16)	0.165	69 (7.1)	29.80 (22.32–37.28)	0.014	0.69 (0.51–0.95)	0.032
GC/GC + GC/AT	304 (92.1)	18.93 (16.26–21.61)		Ref.		903 (92.9)	18.93 (17.53–20.34)		Reference	
Additive	NA	NA	NA	0.77 (0.63–0.95)	0.015	NA	NA	NA	0.82 (0.72–0.92)	0.001

†Survival derived from Kaplan–Meier analysis. ‡Hazard ratios (HRs), 95% confidence intervals (CIs) and their corresponding *P* values were calculated using univariate Cox proportional hazard models. Adeno, adenocarcinoma; aHR, adjusted hazard ratio; ECOG PS, Eastern Cooperative Oncology Group performance status; GP/GC, carboplatin or cisplatin plus gemcitabine; m, months; NP/NC, carboplatin or cisplatin plus vinorelbine; OS, overall survival; $P_{L\text{-}R}$, Log-Rank *P*; SQC, squamous cell carcinoma; TNM, tumor node metastasis; TP/TC, carboplatin or cisplatin plus paclitaxel.

Table 5 Multivariate Cox regression analysis of prognostic factors for overall survival in pooled populations

Variables	HR (95% CI)	*P*
Age (≥58 vs. <58)	1.25 (0.96–1.55)	0.062
TNM stage (IV vs. III)	1.30 (1.11–1.51)	0.004
Tumor histology		0.022
Adeno	Ref.	
SQC	1.22 (1.01–1.47)	0.041
Others	1.38 (1.12–1.71)	0.013
P73 G4C14-to-A4T14		
Dominant	0.82 (0.70–0.97)	0.017
Recessive	0.75 (0.54–1.04)	0.086

All of the variables yielding *P* values < 0.1 in the univariate analysis were used for multivariate Cox regression analysis. Adeno adenocarcinoma; CI, confidence interval; HR, hazard ratio; SQC squamous cell carcinoma; TNM, tumor node metastasis.

be considered with caution. Liu *et al.*'s study was the only one to investigate the association between the *p73*G4C14-to-A4T14 polymorphism and the response of lung adenocarcinoma cell lines to chemotherapy.[26] Nevertheless, the authors also considered that the negative results should be validated further in a prospective study with a larger group of patients. In our opinion, the in vitro assay may not be adequate to simulate the function of chemotherapy drugs in tumor patients. In our study, we found a significant correlation between the *p73* G4C14-to-A4T14 polymorphism and survival outcomes in a Chinese population of advanced NSCLC patients treated with platinum-based chemotherapy. Patients with AT/AT and GC/AT genotypes had a more favorable response and better overall survival than those with the GC/GC genotype, which is consistent with previous published results. This may be explained by variation from the GC to AT allele, leading to the formation of a stem-loop structure, thus modulating the translation efficiency of *p73* in tumors.

The present study has several strengths. We used two relatively large cohorts from four independent oncological departments for the discovery and validation of the association between the *p73* G4C14-to-A4T14 polymorphism and clinical outcomes in advanced NSCLC patients. To ensure relatively homogeneous treatment, only those subjects who did not receive surgery and radiation therapy were enrolled. In addition, we also evaluated this polymorphism classified by models including genotypic, dominant,

Table 6 Association between *p73* genotypes and OS stratified by selected variables

	GC/GC		GC/GC + AT/AT		aHR (95% CI)†		
Variables	N (%)	OS (95% CI) (m)‡	N (%)	OS (95% CI) (m)‡	GC/GC	GC/GC + AT/AT	*P*†
Age (years)							
<58	305 (60.5)	19.93 (17.61–22.25)	199 (39.5)	23.33 (20.07–26.60)	1.00	0.90 (0.72–1.11)	0.305
≥58	285 (60.9)	15.07 (13.31–16.83)	183 (39.1)	22.40 (18.09–26.71)	1.00	0.70 (0.56–0.86)	0.001
Gender							
Male	422 (61.2)	15.97 (14.15–17.78)	268 (38.8)	21.73 (18.88–24.59)	1.00	0.78 (0.66–0.94)	0.007
Female	168 (59.6)	19.27 (16.17–22.37)	114 (40.4)	22.80 (19.38–26.22)	1.00	0.83 (0.55–1.18)	0.125
Smoking history							
Non-smokers	236 (58.4)	19.07 (16.33–21.80)	168 (41.6)	23.57 (20.11–27.03)	1.00	0.81 (0.64–1.02)	0.078
Smokers	354 (62.3)	15.90 (13.92–17.88)	214 (37.7)	22.30 (17.90–26.70)	1.00	0.76 (0.62–0.93)	0.006
ECOG PS							
0–1	535 (60.1)	17.17 (15.44–18.90)	355 (39.9)	23.87 (21.06–26.68)	1.00	0.77 (0.66–0.90)	0.001
2	55 (67.1)	17.77 (9.42–26.12)	27 (32.9)	19.43 (12.59–26.28)	1.00	0.93 (0.60–1.59)	0.890
Chemotherapy							
NP/NC	208 (59.3)	16.27 (13.06–19.48)	143 (40.7)	20.40 (15.01–25.79)	1.00	0.89 (0.69–1.15)	0.371
GP/GC	157 (58.4)	17.47 (14.98–19.95)	112 (41.6)	25.37 (20.18–30.56)	1.00	0.75 (0.56–1.01)	0.055
TP/TC	185 (64.5)	17.97 (14.41–21.52)	102 (35.5)	22.27 (17.82–26.71)	1.00	0.79 (0.60–1.04)	0.091
DP/DC	40 (61.5)	18.40 (12.83–23.97)	25 (38.5)	23.33 (11.22–35.45)	1.00	0.71 (0.37–1.38)	0.313
TNM stage							
III	237 (62.2)	19.10 (16.81–21.40)	144 (37.8)	24.10 (20.16–28.04)	1.00	0.79 (0.62–1.01)	0.059
IV	353 (59.7)	15.20 (13.18–17.22)	238 (40.3)	21.73 (17.69–25.78)	1.00	0.77 (0.63–0.93)	0.008
Tumor histology							
Adeno	370 (59.9)	18.10 (16.02–20.19)	248 (40.1)	25.80 (21.81–29.79)	1.00	0.74 (0.62–0.90)	0.002
SQC	133 (62.4)	14.57 (11.04–18.09)	80 (37.6)	21.87 (14.93–28.81)	1.00	0.76 (0.55–1.06)	0.108
Others	87 (61.7)	16.40 (12.90–19.90)	54 (38.3)	18.30 (11.54–25.07)	1.00	0.95 (0.71–1.26)	0.797

†Hazard ratios (HRs), 95% confidence intervals (CIs) and their corresponding *P* values were calculated using multivariate Cox proportional hazard models, adjusted for all clinical factors. ‡Survival derived from Kaplan–Meier analysis. Adeno, adenocarcinoma; aHR, adjusted hazard ratio; ECOG PS, Eastern Cooperative Oncology Group performance status; GP/GC, carboplatin or cisplatin plus gemcitabine; m, months; NP/NC, carboplatin or cisplatin plus vinorelbine; OS, overall survival; $P_{L\text{-}R}$, Log-Rank *P*; SQC, squamous cell carcinoma; TNM, tumor node metastasis; TP/TC, carboplatin or cisplatin plus paclitaxel.

Table 7 Correlations of *p73* genotypes with chemotherapy efficacy in pooled populations

	ORR (CR + PR)					DCR (CR + PR + SD)				
Genotypes	N (%)	χ^2	*P**	OR (95% CI)†	*P*†	N (%)	χ^2	*P**	OR (95% CI)†	*P*†
GC/GC	87 (14.8)	4.272	0.039	Reference		468 (79.3)	1.122	0.294	Reference	
GC/AT+ AT/AT	76 (19.9)			0.69 (0.41–0.93)	0.047	293 (76.7)			0.92 (0.64–1.45)	0.374

**P* values derived from χ^2 test. †Odds ratios (ORs), 95% confidence intervals (CIs) and their corresponding *P* values were calculated using multivariate logistic regression analysis, adjusted for all clinical factors. CR, complete response; DCR, disease control rate; ORR, objective response rate; PR, partial response; SD, stable disease.

recessive, and additive. However, we acknowledge that there are several limitations. First is the retrospective nature of the study. Second, further investigations of the mechanism behind this polymorphism and potential functions need to be conducted. Third, because the *p73* gene has two alternative splicing transcripts, including TAp73 and ΔNp73, understanding the distribution of different isoforms may help to illuminate the role of the *p73* gene in different types of cancers.

In conclusion, our findings indicated that the *p73* G4C14-to-A4T14 polymorphism may be related to survival outcomes in advanced NSCLC patients following platinum-based chemotherapy. However, further studies are required to investigate the underlying mechanism by which this common *p73* SNP affects outcomes in advanced NSCLC patients.

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Disclosure

No authors report any conflict of interest.

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